Annals of Botany **105**: 737–745, 2010
doi:10.1093/aob/mcq036, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

Sources of variation in self-incompatibility in the Australian forest tree, *Eucalyptus globulus*

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Received: 31 August 2009 Returned for revision: 16 October 2009 Accepted: 20 January 2010 Published electronically: 12 March 2010

• *Background and Aims* One of the major factors affecting the outcrossing rate in *Eucalyptus globulus* is thought to be the inherent self-incompatibility (SI) level of the female tree. SI in this species is mainly due to late-acting pre- and post-zygotic mechanisms operating in the ovary, and not S alleles. This study aimed to assess the phenotypic variation in SI levels within *E. globulus* and determine its genetic control and stability across pollination techniques, sites and seasons.
• *Methods* SI levels were estimated for 105 genotypes originating from across the geographical range of *E. globulus* over multiple years of crossing. Separate grafted trees of some genotypes growing at the same and different sites allowed the genetic basis of the variation in SI to be tested and its stability across sites and seasons to be determined. The SI level of a tree was measured as the relative reduction in seeds obtained per flower pollinated following selfing compared with outcross pollinations. Thus, if seed set is the same, SI is 0 %, and if no self seed is set, SI is 100 %.
• *Key Results* The average SI in *E. globulus* was 91 % and genotypes ranged from 8 to 100 % SI. Most genotypes (>75 %) had SI levels >90 %. There were highly significant differences between genotypes and the within-site broad-sense heritability of percentage SI was high ($H^2 = 0{\cdot}80 \pm 0{\cdot}13$). However, there was evidence that growing site, and to a lesser extent season, can affect the expression of SI levels. Trees with low reproductive loads produced relatively more seed from selfed flowers.
• *Conclusions* There is a strong genetic basis to the phenotypic variation in SI in *E. globulus* within a site. However, the level of SI was affected, but to a lesser extent, by the environment, which in part may reflect the higher probability of selfed zygotes surviving on sites or in seasons where competition for resources is less.

Key words: Heritability, plasticity, resource allocation, SI, mating systems, forest tree.

INTRODUCTION

Mixed mating systems occur frequently in seed plants (Goodwillie *et al.*, 2005), because the ability to set seed after selfing is an advantage when mating partners or pollinators are scarce (Darwin, 1876; Kalisz *et al.*, 2004). Twenty per cent of plant species are estimated to be highly selfing and a further 33 % are intermediate between selfing and outcrossing (Vogler and Kalisz, 2001). However, the vegetative and reproductive fitness of selfed individuals is often low when compared with that of those that are outcrossed (i.e. inbreeding depression; Husband and Schemske, 1996). Therefore, numerous mechanisms have evolved to prevent or reduce self-fertilization in flowering plants, including monoecy, dioecy, dichogamy, male sterility, post-zygotic embryo abortion and self-incompatibility (SI; Sedgley and Griffin, 1989; Sedgley, 1994; Nasrallah *et al.*, 2004).

The most widespread SI system is homomorphic SI, estimated to occur in 81 families (of 382 angiosperm families assessed) (Clarke and Newbigin, 1993; Barrett and Cruzan, 1994). In species with homomorphic SI, self-pollination is prevented by the arrest of self-pollen tube growth prior to fertilization. This system is usually controlled by genes at the S locus, each of which has multiple alleles (Hiscock and McInnis, 2003). SI systems that operate in the ovary have been considered rare, and remain ill defined (Seavey and Bawa, 1986; Sage *et al.*, 1994). However, the number of studies that have reported such late-acting SI has increased, with studies across a number of families including Asclepiadaceae, Bignoniaceae, Bombacaceae, Leguminosae, Myrtaceae, Polemoniaceae and Winteraceae (Seavey and Bawa, 1986; Barrett, 1988; Gibbs and Bianchi, 1993, 1999; Gibbs *et al.*, 1999; Lipow and Wyatt, 2000; Pound *et al.*, 2003*a*, *b*; Sage and Sampson, 2003; Bianchi *et al.*, 2005; Bittencourt and Semir, 2006; Sage *et al.*, 2006; Ghazoul and Satake, 2009). Late-acting SI is thought to occur when fruits fail to develop following selfing, despite self-pollen tubes growing normally down the style (Gibbs and Bianchi, 1999). Late-acting systems can be divided into three types: (1) ovarian inhibition of incompatible pollen tubes before the ovule is reached; (2) pre-fertilization inhibition of the ovule (male gametes are released into the embryo sac but fertilization does not occur); and (3) post-zygotic rejection (Seavey and Bawa, 1986). However, discrimination between active post-zygotic rejection of selfed embryos and early-acting inbreeding depression arising from the expression of embryonic lethal genes is difficult (Seavey and Bawa, 1986), and in

the absence of a mechanism both processes tend to be incorporated under the term late-acting SI (Ghazoul and Satake, 2009). The wastage of ovules by late-acting SI mechanisms could be considered maladaptive; however, if the plant produces an excess of flowers and ovules, then an advantage could include the ability for the maternal parent to select the best pollen genotypes, thus allowing flexibility in the choice of parents (Seavey and Bawa, 1986; Waser and Price, 1991). Although late-acting SI systems have not been as widely examined as mechanisms that occur at the stigma, there have been studies that suggest they are under genetic control (Cope, 1962; Lipow and Wyatt, 2000).

Trees of the genus *Eucalyptus* have a mixed mating system, although most are preferentially outcrossing (Griffin *et al.*, 1987; Eldridge *et al.*, 1993), and the setting of selfed seed can lead to inferior progeny and inbreeding depression (Hardner and Potts, 1995, 1997; Hardner *et al.*, 1998). The mechanism of SI has been studied in only a few species of *Eucalyptus*; however, these studies have revealed that both pre- and post-zygotic mechanisms operate (Sedgley *et al.*, 1989; Sedgley and Smith, 1989; Ellis and Sedgley, 1992; Sedgley and Granger, 1996; Pound *et al.*, 2002*a*, *b*, 2003*a*, *b*; Horsley and Johnson, 2007).

Eucalyptus globulus (tasmanian blue gum) is the premier pulpwood plantation eucalypt for temperate regions of the world (Eldridge *et al.*, 1993; Potts *et al.*, 2004, 2008). As it is often difficult to propagate *E. globulus* clonally (Tibbits *et al.*, 1997), open-pollinated seed orchards have been established for the production of improved seed. In *E. globulus*, outcrossing rates have been reported to vary between 41 and 100 % at the individual tree level under open pollination (Hardner *et al.*, 1996; Patterson *et al.*, 2000), and at an orchard level from 77 % (Moncur, 1995) to as low as 60 % (Patterson *et al.*, 2004*b*). Low outcrossing rates in seed orchards is of concern as selfing results in inbreeding depression which has a severe impact on the productivity of *E. globulus* trees in plantations (Hardner and Potts, 1995; Hardner *et al.*, 1998; Potts *et al.*, 2008). The study of the factors most likely to affect outcrossing rates in an *E. globulus* seed orchard by Patterson *et al* (2004*b*) included: seasonal variation in flowering time, flower abundance, canopy position and SI level. Outcrossing rates were significantly higher in the upper than in the lower parts of tree canopies of self-compatible trees. However, neither flowering time nor the flower abundance of individual trees had a consistent effect on their outcrossing rates (Patterson *et al.*, 2004*b*). Rather, SI level (% SI) was identified as the key factor driving variation in outcrossing rates. This result raised the possibility of selecting self-incompatible trees to increase outcrossing rates in seed orchards, thereby improving gains in plantations by reducing the effects of inbreeding depression. In order to implement such a strategy, an understanding of the variation and genetic control of the % SI in *E. globulus* was required. The present study combined data from 20 years of controlled pollination of *E. globulus* trees in seed orchards and trees from native stands to determine the phenotypic variation in % SI, and used graft-replicated genotypes in seed orchards to study the genetic control and the stability of % SI across seasons, sites and pollination techniques.

MATERIALS AND METHODS

Measurement of % SI and pollination techniques

Self-incompatibility (SI) of an individual of *Eucalyptus globulus* Labill. was defined as the per cent reduction in seed set after selfing compared with outcrossing (Pound *et al.*, 2002*b*). In the present case, it was calculated as:

$$\%\ \mathrm{SI} = \frac{(\mathrm{OUTCROSS} - \mathrm{SELF})}{\mathrm{OUTCROSS}} \times 100$$

where OUTCROSS is the number of viable seed per flower produced from controlled pollination with outcross pollen, and SELF is the number of viable seed per flower from controlled pollination with self-pollen (Lloyd, 1965; Pound *et al.*, 2002*a*, *b*). It requires both self- and outcross pollinations to be undertaken on the same tree. Open-pollinated (OP) depression was calculated as the per cent reduction in seed per flower after open pollination compared with outcross pollination (similar to the calculation of % SI except replacing SELF with OP seed set per flower).

Two methods of controlled pollination were used to estimate % SI in *E. globulus*, the three-visit method and the single-visit method. The three-visit pollination technique required separate visits to a tree for: (*a*) emasculation at operculum lift to prevent self-fertilization and isolation of the flower with a bag to exclude other pollen; (*b*) pollination a week later when the stigma was receptive; and (*c*) removal of the bag 3–4 weeks later (Tibbits, 1986; Moncur, 1995). The more recently developed one-stop (Harbard *et al.*, 1999) or single-visit (Williams *et al.*, 1999) pollination technique reduced the operation to a single visit to the female tree. As in the three-visit method, anthers were removed (emasculation) at operculum lift, and then the top 5–10 % of the style (including the stigma) was removed with a scalpel, causing the cut surface to become sticky and receptive. Pollen was then applied and finally the flower was isolated with a bag, piece of plastic tube or balloon.

In general, crossing was undertaken using 10–20 flowers for each outcrossing and self-treatment on a tree in order to obtain robust estimates. On each tree, outcrosses were produced using five different viable male pollens (usually four flowers per male) each from a different race of *E. globulus* [Southern Tasmania (South Geeveston or Taranna locality), Strzelecki Ranges, Furneaux, South-eastern Tasmania (Hobart locality) and Western Otways Ranges; see Dutkowski and Potts (1999)]. In some experiments, 10–20 flowers at similar developmental stage to the control pollinated flowers were labelled and left to open pollinate. Branches used for crossing were distributed around the tree and, on each branch, four flowers of one outcross pollen were paired with four selfed flowers, and four OP flowers (in some experiments).

Seed capsules were collected approx. 1 year after pollination, dried and seed extracted individually. The *E. globulus* capsule contains viable seeds, aborted fertilized seeds and chaff (Drake, 1975; Boland *et al.*, 1980), all of which are found in a seed-lot. The number of viable (filled seeds with black testa) and inviable (flattened seeds with black testa) seed was counted and used to calculate (*a*) the number of viable seed per capsule; (*b*) viable seed per flower pollinated; and (*c*) percentage of inviable seed (Hardner and Potts, 1995).

Capsule set was calculated as the percentage of capsules collected per flower crossed.

Effect of genotypes on variation in seed set and % SI

Controlled pollinations were undertaken over two flowering seasons in a clonal breeding arboretum located near Manjimup, Western Australia (34°15′S, 116°7′E) in 1997 and 1998. Pollinations were completed on a total of 25 ramets from eight genotypes (range 1–4 ramets per genotype), with a total of 317, 312 and 389 flowers selfed, outcrossed and left to open pollinate, respectively, over the two seasons. Six of the genotypes were tested in both years. The controlled crosses were done using the three-visit procedure. On each ramet, generally, ten flowers were pollinated with self-pollen and ten flowers were pollinated with outcross pollen, and 10–26 flowers were labelled and left to open pollinate. Each unique outcross pollen was applied in a separate pollination bag. Different ramets of the same genotype were outcrossed with pollen from the same five races but different genotypes.

The effect of cross type (i.e. open pollination, outcross and self) on seed set traits was analysed using PROC MIXED (SAS version 9·1, SAS Institute Inc.), with cross type, year and their interaction fixed, and genotype, genotype × cross type, genotype × year, and ramet within genotype as random effects. To analyse the variation in seed set traits within each cross type, and to test whether there was a significant difference in OP depression and % SI between the eight genotypes used in this study, a mixed model was also fitted with PROC MIXED, with genotype and year fixed and the random variation between ramets within genotype as the residual term. An estimate of the broad-sense heritability (H^2) was obtained for each trait, treating these factors as random and dividing the between-genotype variance component by the phenotypic variance (Falconer, 1989). The phenotypic variation was estimated as the sum of the genotype, year and residual variance components. Correlations between the OP seed set traits and the selfed or outcrossed seed set traits (percentage capsule set, seed per capsule, seed per flower and percentage of viable seed) were calculated using the Pearson PROC CORR procedure of SAS, at both the ramet (25 trees) and genotype (eight genotypes) level. This procedure was also used to correlate the % SI with the absolute OP seed set traits and the OP depression. In all analyses, $\log_{10}$ transformations were applied to (*a*) the number of viable seed per flower; (*b*) the number of viable seed per capsule; and (*c*) the percentage of inviable seed to optimize the normality of their residuals.

Effects of pollination method, season and site on % SI

To study the effects of pollination method, season and site on % SI, 92 trees across 69 genotypes were screened, with a total of 5289 flowers pollinated in 2001/2002. The trees were located either in native stands or in clonal grafted seed production orchards in Tasmania (north-western and southern Tasmania) and Western Australia (Table 1). All these trees were included in the phenotypic study of % SI discussed later. Separate experiments were designed to test the effect of pollination method, season and site, but all three used the same crossing design as detailed above.

TABLE 1. *Location of trees used in the study of the effects of pollination method, season and site on percentage self-incompatibility in Eucalyptus globulus*

Location	State	Latitude	Longitude	Established	Trees
Arboreta					
Manjimup	WA	34°14′S	116°09′E	1996	50
Ridgley	TAS	41°08′S	145°50′E	1996	28
Massey Greene Drive	TAS	41°05′S	145°54′E	1989	6
Grange	TAS	42°46′S	147°05′E	1997	1
Kingsclere	TAS	42°10′S	145°51′E	1998	1
Native stand	TAS				
Tinderbox	TAS	43°03′S	147°19′E	n/a	6
Total					92

The number of trees crossed at each arboretum and in native stands is indicated. WA, Western Australia; TAS, Tasmania.

To determine whether the fast single-visit pollination method that involved stigma removal could be used to measure % SI rather than the three-visit procedure, ten genotypes were compared using both the single-visit and the three-visit method. Balloons were used to isolate each flower, with all flowers under the same tag pollinated with the same pollen and technique. The crossing design was as mentioned above. A paired *t*-test was used to find whether there was a difference in % SI between the two pollination methods using genotypes as replicates.

To examine the effect of season on % SI, 11 trees (ten genotypes) were screened over 2 years (2001 and 2002) using the single-visit procedure. The trees were located in arboreta at Ridgley (Tasmania) and Manjimup (Western Australia). A total of 847 and 846 flowers were selfed and outcrossed, respectively. A paired *t*-test was used to analyse the differences in % SI between seasons using tree as replicates.

To test if % SI varied with site, 13 genotypes replicated as grafts at two sites were compared in 2001 or 2002 using the single-visit method. Trees at the Ridgley and Manjimup arboreta were used and the crossing design was the same as previously mentioned. Of the paired comparisons, three genotypes were assessed at both sites in 2001, four in 2002 and six involved comparison across sites in different seasons. A score of the number of buds on each tree was undertaken as part of routine breeding operations based on ground level observations of the canopy. Paired *t*-tests were used to analyse the differences in % SI between sites with genotypes treated as replicates.

Phenotypic and regional variation in % SI

Estimates of % SI were compiled for 162 trees (representing 105 genotypes) in seed orchards, breeding arboreta and native stands from crossing undertaken from 1987 to 2004 (Table 2). This included data from the present and previous studies (Potts and Savva, 1988; Hardner and Potts, 1995; Hardner *et al.*, 1998; Pound *et al.*, 2002*a*, *b*) and unpublished data (data provided by Peter Buxton, Grand Ridge Plantations). SI estimates dating to before 1999 used the three-visit pollination technique, and data collected thereafter involved both three-visit and single-visit pollination techniques. When multiple SI

TABLE 2. *The number of* Eucalyptus globulus *trees and genotypes from each region that were screened for their level of self-incompatibility and the years in which the crossing occurred*

Region	No. of trees	No. of genotypes	Years crossed
Furneaux	64	30	1988–2004
Otways	32	24	1997–2002
Strzelecki	31	22	1998–2003
Eastern Tasmania	26	20	1987–2002
King Island	9	9	1988–2001
Total	162	105	

estimates were obtained from the same tree or the same genotype, the mean % SI was calculated. This data set was used to examine, first, the phenotypic variation in *E. globulus* at both the tree and genotype level, and, secondly, the possible differences in % SI between genotypes originating from different geographical regions within the native range of *E. globulus*. This data set combined % SI measurements from genotypes in native stands and selections originating from these regions but growing in seed orchards or arboreta. The effect of native region of origin was analysed using a one-way model in PROC MIXED (SAS version 9·1) with region fitted as a fixed effect.

RESULTS

Variation in seed set and % SI between genotypes

Cross type (open pollination, outcross or self-cross) had a significant effect on (*a*) number of viable seed per capsule; (*b*) number of viable seed per capsule per flower; and (*c*) the percentage of inviable seed per capsule (Table 3). Cross type did not significantly affect capsule set. When compared with outcross pollination, self-pollination significantly reduced overall seed set from 12·5 to 2·0 seed per flower and 20·9 to 3·2 seed per capsule, respectively. Even in comparison with the seed set following open pollination (10·6 seed per flower and 14·5 seed per capsule), the number of seed set after selfing was significantly reduced. Open pollination significantly decreased seed per capsule compared with outcrossing, but capsule abortion was lower, resulting in similar levels of seed per flower obtained for these cross types. Selfing (24 %) and open pollination (18 %) resulted in a significantly higher percentage of inviable seeds than outcrossing (11 %). For all cross types, genotypes were significantly different in seed per flower and seed per capsule following all cross types (Table 3). The genotypes also differed significantly in their capsule set after outcross and self-pollination.

The % SI was significantly different between the genotypes tested ($F_{7,16} = 14{\cdot}3$; $P < 0{\cdot}001$). There was a significant effect of season on % SI ($F_{1,16} = 4{\cdot}6$; $P = 0{\cdot}048$); however, it was relatively small when compared with the difference between genotypes. The genotype % SI ranged from 14·4 to 99·6 %, whereas the variation between seasons was more limited with mean % SI of 80·7 % in 1997 and 85·3 % in 1998. The per cent OP depression was also significantly different between genotypes ($F_{7,16} = 7{\cdot}5$; $P < 0{\cdot}001$); however, there was no effect of season on this trait ($F_{1,16} = 0{\cdot}01$; $P > 0{\cdot}05$). The H^2 of % SI was $0{\cdot}80 \pm 0{\cdot}13$, and that of per cent OP depression was $0{\cdot}73 \pm 0{\cdot}14$.

At the ramet level, OP seed per flower was positively correlated with seed per flower under outcrossing ($r_{23} = 0{\cdot}59$; $P < 0{\cdot}01$). However, while positive, the correlation was not significant at the genotype level ($r_6 = 0{\cdot}34$; $P = 0{\cdot}41$). The number of viable seed in OP capsules was not significantly correlated with the % SI of the ramet ($r_{23} = -0{\cdot}06$, $P = 0{\cdot}76$) or genotype ($r_6 = -0{\cdot}33$, $P = 0{\cdot}43$). However, the level of OP depression was significantly and positively correlated with the % SI for seed per flower ($r_6 = 0{\cdot}90$; $P < 0{\cdot}01$). In other words, the more self-incompatible a tree, the greater the reduction in seed per flower after open pollination in comparison with seed per flower after outcross pollination.

Effects of pollination method, season and site on % SI

The mean SI across all genotypes for the three-visit pollination method was 87·1 % and for the single-visit pollination method 89·0 %. There was no significant difference between the two methods (paired $t_9 = -0{\cdot}38$, $P = 0{\cdot}71$). This result therefore allowed the merging of data from both pollination techniques. There was no effect of season on the % SI of the 11 trees tested (paired $t_{10} = -1{\cdot}65$, $P = 0{\cdot}27$). The mean % SI in 2001 was 96·0 % while in 2002 it was 97·9 %, whereas genotype means, averaged across sites, ranged from 72·9 to

TABLE 3. *Variation in cross type and genotype seed set in the* Eucalyptus globulus *crossing experiment*

	Between cross types					Between genotypes					
	OP	Outcross	Self	*F*-value	*P*	OP		Outcross		Self	
						F-value	*P*	*F*-value	*P*	*F*-value	*P*
% capset	73[a]	65[a]	55[a]	2·1	0·161	2·1	0·102	2·7	0·036	2·7	0·044
Viable seed per flower	10·6[a]	12·5[a]	2·0[b]	18·6	<0·001	4·7	0·004	3·0	0·025	5·4	0·001
Viable seed per capsule	14·5[b]	20·9[a]	3·2[c]	26·0	<0·001	6·2	0·001	3·5	0·016	6·9	0·001
% inviable seed	18[a]	11[b]	24[a]	8·7	0·004	0·7	0·696	0·9	0·547	1·9	0·160

This table includes untransformed mean percentage of capsules set (% capset), viable seed per flower (seed/flower), viable seed per capsule (seed/capsule) and the percentage of inviable seed (% inviable seed) for the three different cross types: open pollinated (OP), outcross and self.

F-values and their probability are shown and were based on the transformed data. For the test of the difference between cross types ($d.f._{cross\ type} = 2$, $d.f._{residual} = 14$) and the difference between the eight genotypes under the different pollination types ($d.f._{genotype} = 7$, $d.f._{residual} = 12–20$ 0, respectively). Common letters within each cross type indicate treatment means are not significantly different ($P < 0{\cdot}05$) based on the Tukey–Kramer adjustment.

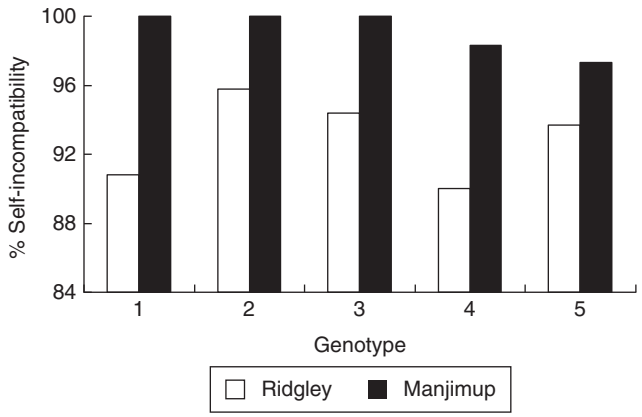

FIG. 1. Site had a significant effect on level of SI in *Eucalyptus globulus*. Eight of the genotypes exhibited no difference in % SI, i.e. they were 100 % self-incompatible at both sites. However, the remaining five genotypes shown above had lower SI when measured at Ridgley than when measured at Manjimup.

100 %. There was a significant effect of site on % SI when the same genotypes were tested in the two orchards used in this study (paired $t_{12} = 2{\cdot}49$, $P = 0{\cdot}03$). The mean % SI for the Manjimup orchard in Western Australia was slightly higher (99·7 %) than the mean for the Ridgley orchard in Tasmania (97·3 %). Of the 13 genotypes tested in both locations, eight exhibited 100 % SI at both Manjimup and Ridgley. The remaining five genotypes had higher SI values in Manjimup than in Ridgley (Fig. 1). In each case where there was a difference in % SI between the two orchards; the number of flower buds per tree was always higher at Manjimup than at Ridgley (data not shown).

Phenotypic and regional variation in % SI

Eucalyptus globulus genotypes ranged in their % SI from highly self-compatible (7·9 %) to completely self-incompatible (100 %; Fig. 2). The average SI in *E. globulus* was 91 %. The distribution of % SI was skewed such that the majority (75 %) of the genotypes had a % SI >90 %, and 33% had a % SI of 100 %. It should be noted that these estimates confound different pollinating methods, seasons and sites. There was no significant phenotypic difference in % SI between *E. globulus* originating from the different geographical regions ($F_{4,100} = 0{\cdot}65$; $P = 0{\cdot}63$). However, there was a trend for trees from mainland Australia (Strzelecki and Otways) to have higher % SI than trees from Eastern Tasmania and King Island (Table 4).

DISCUSSION

Consistent with previous studies of *E. globulus* (Hardner and Potts, 1995; Hardner *et al.*, 1998; Pound *et al.*, 2002*b*) a significant reduction in seed set following self-pollination when compared with outcross pollination was found. The lower number of seed per capsule following open pollination compared with outcross pollination could have been due to either a lower overall pollen load or an increased self-pollen load that could lead to subsequent abortion of selfed zygotes. The proportion of inviable seed was significantly higher after selfing and open pollination. This is most probably due to the late-acting SI mechanism of *E. globulus*, with post-zygotic abortion of self-fertilized ovules (Pound *et al.*, 2002*a*). The significant difference between genotypes in capsule set, seed per flower and seed per capsule after outcross and self-pollination is an indication of the variation in SI between the genotypes or variation in the reproductive success of a genotype *per se*. The high level of correlation between the different cross types for the proportion of viable seed indicates that *E. globulus* trees are inherently variable in this trait.

OP depression in viable seed per flower was significantly variable between genotypes, with a significant positive correlation between % SI and OP depression in seed set. This indicates that seed set after open pollination in comparison with the seed set after outcrossing is reduced in SI trees. Self-compatible trees may have a selective advantage when pollen or pollen vectors are scarce. Mixed mating is almost twice as likely to occur in animal-pollinated species than in wind- or water-pollinated species (Barrett, 2002, 2003; Goodwillie *et al.*, 2005), and may help ensure reproductive output in unpredictable pollinator environments (Kalisz *et al.*, 2004). The peak flowering times of *E. globulus* trees are genetically highly variable between races (Gore and Potts, 1995; Apiolaza *et al.*, 2001) and also within races (Rebecca Jones, pers. comm.), and trees that are self-compatible could be favoured when outcross pollen is unavailable. Another possible advantage which could explain the

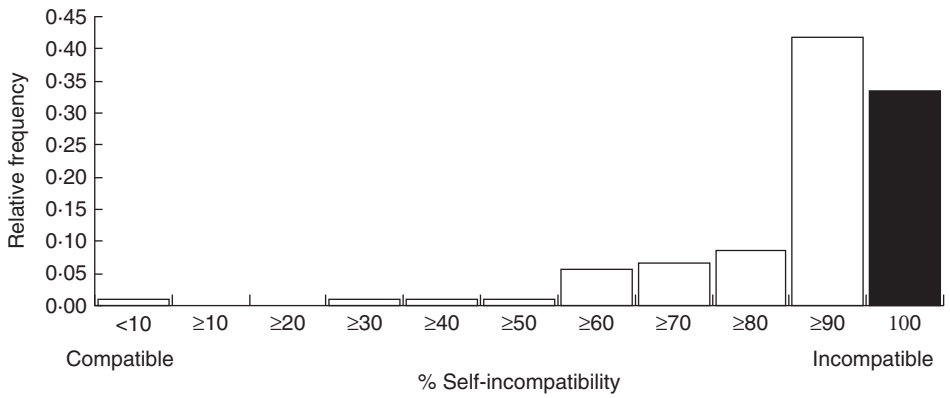

FIG. 2. Relative frequency histogram of the phenotypic variation in the level of self-incompatibility amongst 105 *E. globulus* genotypes. Black = 100 % SI.

TABLE 4. *Self-incompatibility of* Eucalyptus globulus*genotypes originating from five different geographical regions*

Region	No. of genotypes	Mean % SI
Eastern Tasmania	20	87·5
King Island	9	88·6
Furneaux Islands	30	91·2
Otway Ranges	24	91·0
Strzelecki Ranges	22	94·8
Total	105	91·0

The table shows the region of origin, the number of genotypes screened and the mean percentage self-incompatibility (% SI).

maintenance of mixed mating systems is the predator satiation hypothesis (Ghazoul and Satake, 2009). Under this hypothesis it is argued that selfed seeds that normally abort at a relatively late developmental stages may serve an important function in diluting the impact of pre-dispersal seed predators.

This study provides evidence that variation in % SI is under strong genetic control and has a high H^2 (0·80). While there have been studies of the individual gene control of early acting SI in plants (Ehrenreich and Purugganan, 2006), few have reported the quantitative genetic basis to the variation within a species (Stephenson *et al.*, 2000). Variation in % SI occurs in many eucalypt species, and a genetic basis to the reported late-acting SI mechanism in several species has been hypothesized (James and Kennington, 1993; Pound *et al.*, 2002*b*). However, the present study is the first to report a significant genetic basis to this variation in % SI.

The use of the more efficient single-visit pollination method (rather than the original three-visit procedure) did not significantly affect estimates of % SI in *E. globulus*. This allows for the comparison of % SI derived from both controlled pollination methods. More importantly, the single-visit pollination method significantly reduces the screening time (and therefore cost) of *E. globulus* trees for % SI (Williams *et al.*, 1999). Removal of the tip of the style as in the single-visit pollination technique was considered unlikely to alter the level of SI, as the mechanism for SI in *E. globulus* is late acting (Pound *et al.*, 2002*a*, *b*, 2003*a*). There is no evidence for pollen competition between self-pollen and outcross pollen in the style of *E. globulus*, with both self-pollen and outcross pollen tubes successfully reaching and fertilizing the ovules (Pound *et al.*, 2003*a*). However, there are both pre- and post-zygotic barriers to selfing in *E. globulus*, with a slight reduction in ovule penetration by self-pollen tubes, and significantly reduced seed set after selfing (Pound *et al.*, 2002*b*). It is thought that the major mechanism of SI occurs after ovule penetration by self-pollen tubes, such as failed or incomplete fertilization or abortion after self-fertilization has occurred (Pound *et al.*, 2002*a*, *b*). This is consistent with the present results showing removal of the upper style including the stigma does not affect the expression of % SI. Horsley and Johnson (2007) reported that self-pollen tubes grew more slowly than outcross pollen tubes in *E. grandis* and *E. urophylla*, which could accentuate the selection against self-pollinations under mixed mating. However, there is no evidence that this is a significant factor in *E. globulus*, since the relative success of self-pollen under mixed pollinations directly reflected that predicted from the % SI of the mother in the absence of competition (Pound *et al.*, 2003*a*).

In the present study, the % SI of a genotype did vary across sites. The same genotypes were measured at an orchard in Tasmania and in Western Australia. On average, the genotypes at the Western Australian orchard were slightly more self-incompatible than the same in Tasmania. These genotypes also had higher seed per capsule after outcross pollination and much higher bud numbers in Western Australia than the same genotype in Tasmania. Competition for resource allocation could account for the difference in SI level between sites. Resource competition is thought to drive the early abortion of capsules with low seed set in *E. globulus* (Suitor *et al.*, 2008), and site differences in thresholds for abortion (Suitor *et al.*, 2008). It is likely that there was increased competition for resources on the Western Australian trees, with their higher bud numbers per tree and higher seed per capsule. Under this scenario, it is suggested that the outcrossed buds would attract more resources and develop into mature fruit, whereas the developing selfed capsules, which have much lower seed numbers, would be more likely to abort. Conversely, in the Tasmanian orchard, the level of competition for resources between developing capsules on the tree would be lower, as there were fewer buds and lower seed per capsule, allowing more selfed buds to develop to maturity. Resource allocation has been suggested to be the cause of ovule abortion in other eucalypt species including *E. camaldulensis* (James and Kennington, 1993), *E. spathulata*, *E. platypus* (Sedgley and Granger, 1996) and *E. regnans* (Griffin *et al.*, 1987).

A hormonally controlled positive feedback system has been suggested to explain fruit abortion in plants (Wiens *et al.*, 1987). As ovules mature, they produce growth regulators, which stimulate fruit development and direct resources to developing fruit (Stephenson, 1981). Therefore, fruit with a greater number of developing ovules would generate a stronger 'sink' for maternally allocated resources, and outcompete those with fewer developing ovules, particularly if resources are limited (Wiens *et al.*, 1987). Furthermore, James and Kennington (1993) suggested that selection between developing selfed and outcrossed seed occurs within the capsules of *E. camaldulensis* and that this post-zygotic selection was dependent on trees carrying a large number of deleterious recessive alleles that cause seed abortion in the homozygous condition. It is possible that within self-incompatible *E. globulus* trees, there is competition between selfed and outcrossed zygotes, with the less fit more homozygous selfed progeny unable to compete with the more heterozygous outcrossed progeny for maternal resources. At times when there is less competition for resources (fewer buds on a tree) then the selfed progeny, despite their inferior genotype, could survive and become seed.

There was a slight effect of season on % SI in the 1997/1998 study, but none detected in the 2001/2002 study. Resources available for fruit production could vary between years depending on weather conditions and the levels of herbivory and disease. The total number of developing fruit drawing upon maternal resources could also vary between seasons (Stephenson, 1981). While the numbers of flower buds on the trees in the 1997/1998 study were not available, it is

possible that, as discussed above, the variation in % SI could be due to seasonal differences in competition for resources between the selfed and outcrossed buds for attracting resources. Nevertheless, the seasonal effects were small compared with the differences detected between the genotypes in the 1997/1998 study. The relative stability of % SI across seasons is consistent with the stability of outcrossing rates reported in *E. globulus* (across four flowering seasons; McGowen *et al.*, 2004).

SI was phenotypically variable between *E. globulus* trees and genotypes, with the majority of trees relatively self-incompatible (SI >90 %). Variation in % SI has been reported in other plant species, including *Flourensia vernua* (Ferrer *et al.*, 2009); however, in *E. globulus*, the majority of plants were highly self-incompatible. Variation in % SI has also been reported in the Myrtaceae family, with both self-compatible and self-incompatible trees existing (Potts and Savva, 1988; Beardsell *et al.*, 1993*a*, *b*; Proenca and Gibbs, 1994; Nic Lughadha and Proenca, 1996; Schmidt-Adam *et al.*, 1999). Variation in % SI has been reported in other eucalypt species including *E. regnans* (Eldridge and Griffin, 1983; Griffin *et al.*, 1987; Sedgley *et al.*, 1989), *E. grandis* (Hodgson, 1976*a*, *b*), *E. nitens* (Tibbits, 1989), *E. gunnii* (Potts and Cauvin, 1988), *E. pulverulenta* (Pryor, 1976) and *E. cladocalyx* (Ellis and Sedgley, 1992). However, all these studies involved few trees, and this is the first large-scale study of the distribution of phenotypic variation in % SI in *Eucalyptus*. Despite expectations (Barrett and Kohn, 1991; Reinartz and Les, 1994; Igic *et al.*, 2008) that the isolated King Island population would have a lower % SI than the other *E. globulus* populations studied, a significant difference between regions was not detected.

The genetic basis to SI and OP seed depression in *E. globulus* has important implications in seed orchard management, allowing the exploitation of these traits to improve seed quality after open pollination and mass supplementary pollination. Seed collectors could be reasonably confident that if they collected seed from a tree with 100 % SI it would be completely outcrossed under open pollination. This would considerably reduce the costs of producing genetically superior seed. Even though the decrease in seed set after open pollination (OP seed depression) in these trees may counterbalance the economic benefits, it would not be significant in comparison with the costs of control pollination. Similarly, in mass supplementary pollination it could be possible to reduce further the contamination from self-pollen, which is reported to be as low as 4·8 % (Patterson *et al.*, 2004*a*), if trees with 100 % SI were selected for pollination. However, trees which are self-compatible should be retained in seed orchards to maintain a genetically diverse and abundant pollen environment (Patterson *et al.*, 2004*b*). Research is required to ensure that selection for high SI will not adversely affect outcross progeny performance. It is theoretically possible that growth of outcrossed progeny will be depressed if both parents have a high % SI. This could occur if the SI mechanism depends on the expression of numerous deleterious recessive alleles in the embryos, such that selfed embryos abort when homozygous for several of these alleles (Sedgley, 1994), but only if the deleterious alleles are expressed at later stages in life due to incomplete dominance (Fu, 1994).

ACKNOWLEDGEMENTS

This research was supported by Australian Research Council Grants (C19906699 and LP0016752), the CRC for Sustainable Production Forestry and the STBA. We thank Peter Gore of SeedEnergy and Peter Buxton of Hancocks for provision of data, and WAPRES and Gunns Ltd for access to arboreta.

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